CLAIMS

What is claimed is:

1. A method of screening a substance of interest for heme independent inhibition of soluble guanylyl cyclase comprising:

a) obtaining purified $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase enzyme or a cell lysate containing $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase enzyme;

b) assaying said purified enzyme or cell lysate for formation of cGMP from GTP in the presence of said substance;

c) assaying said purified enzyme or cell lysate for formation of cGMP from GTP in the absence of said substance;

d) optionally, carrying out steps b) and c) in the presence or absence of an activator; and

e) comparing the results from b) and c), and, d), if present, to determine whether said substance inhibits cGMP production by said purified enzyme or cell lysate.

2. A method of screening a substance of interest for heme independent activation of soluble guanylyl cyclase comprising:

a) obtaining purified $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase enzyme or a cell lysate containing $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase enzyme;

b) assaying said purified enzyme or cell lysate for formation of cGMP from GTP in the presence of said substance;

c) assaying said purified enzyme or cell lysate for formation of cGMP from GTP in the absence of said substance;

d) optionally, carrying out steps b) and c) in the presence or absence of an activator other than said substance of interest; and

e) comparing the results from b) and c), and, d), if present, to determine whether said substance enhances cGMP production by said purified enzyme or cell lysate.

3. A method of identifying a functional region of soluble guanylyl cyclase that is responsible for sGC regulation comprising:

a) obtaining a library of deletion mutants of α subunit of soluble guanylyl cyclase;

b) producing mutant sGC enzymes containing β^{Cys105} subunit and α subunits with deletions obtained in step a);

c) obtaining cell lysates comprising the respective mutant sGC enzymes with α subunit deletions, from step b);

d) optionally, purifying said mutant sGC enzymes from step c);

e) assaying said purified enzymes or cell lysates from step c) or d) for formation of cGMP from GTP in the absence of activators or inhibitors;

f) assaying purified wild type sGC enzyme, or a cell lysate comprising said wild type sGC enzyme, for formation of cGMP from GTP in the absence of activators or inhibitors;

g) assaying purified $\alpha\beta^{Cys105}$ mutant sGC enzyme, or a cell lysate comprising said $\alpha\beta^{Cys105}$ sGC enzyme, for formation of cGMP from GTP in the absence of activators or inhibitors;

h) comparing the results from e) and f), and g) to determine whether any said α subunit deletion decreases or increases the activity of the corresponding mutant enzyme tested in step e), as compared to the $\alpha\beta^{Cys105}$ mutant sGC enzyme in step g), to levels comparable or identical to that of the wild type sGC enzyme in step f);

i) using the results of the comparison in step h), identifying an α subunit deletion mutant from step a) containing a deletion mutation that effects sGC activation.

4. The method of claim 3 wherein step i) comprises identifying an α subunit deletion mutant from step a) containing a deletion mutation that is critical for sGC activation.

5. A method to aid in identifying structural features of soluble guanylyl cyclase stimulation comprising

crystallizing purified $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase enzyme in the presence of DTT;

crystallizing purified $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase enzyme in the absence of DTT; and

comparing the resulting soluble guanylyl cyclase enzyme crystals, and

determining structural changes in the soluble guanylyl cyclase protein associated with the presence or absence of DTT.

6. A method of increasing and/or sustaining intracellular production of cyclic GMP in a mammalian cell comprising:

providing $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase, or the β^{Cys105} subunit thereof, to said cell, and/or

constitutively expressing in said cell of the $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase gene, or a portion thereof containing at least the DNA coding for the β^{Cys105} subunit.

7. A method of treating or preventing a mammalian pathophysiologic condition associated with cyclic GMP regulation of a cellular process, the method comprising:

increasing and/or sustaining intracellular production of cGMP by constitutively expressing $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase, or

inhibiting cGMP production by administering an inhibitor of soluble guanylyl cyclase that acts independently of the heme moiety of soluble guanylyl cyclase, in a mammal in need of such treatment or prevention.

8. The method of claim 7 wherein increasing and/or sustaining cGMP production comprises delivering $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase enzyme, or the β^{Cys105} subunit thereof, to at least one cell in said mammal.

9. The method of claim 7 wherein increasing and/or sustaining cGMP production comprises delivering the $\alpha\beta^{Cys105}$ mutant soluble guanylyl cyclase gene, or the β^{Cys105} subunit portion thereof, to at least one cell in said mammal.

10. The method of claim 7 wherein treating or preventing a mammalian pathophysiologic condition associated with cGMP regulation of a cellular process comprising treating or attenuating angina.

11. The method of claim 7 wherein said pathophysiologic condition comprises cardiovascular disease.

12. The method of claim 11 wherein said cardiovascular disease is selected from the group consisting of chronic heart disease, chronic hypertension, thrombosis, atherosclerosis, congestive heart failure, and myocardial infarction.

13. The method of claim 7 wherein said pathophysiologic condition comprises a post-angioplasty complication.

14. The method of claim 7 wherein said pathophysiologic condition comprises a complication arising from a vein graft operation.

15. The method of claim 7 wherein treating or preventing a mammalian pathophysiologic condition associated with cGMP regulation of a cellular process comprises treating a tumor or attenuating or preventing tumor metastasis.

16. The method of claim 7 wherein treating or preventing a mammalian pathophysiologic condition associated with cGMP regulation of a cellular process comprising treating or attenuating a penile dysfunction.

17. The method of claim 7 wherein said pathophysiologic condition comprises septic shock.